Esp

Week 7

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Overhang PCR of T7-EB to add BamHI, first attempt

Responsible

Aman Mebrahtu, Oscar Frisell

Protocol Used

PCR protocol

Modification and comments to protocol

Experimental Set Up

Description: The aim of this experiment was to insert the restriction site BamHI into T7-EB by Overhang PCR. Two T7-EB samples were used for this experiment hereafter referred as EB1 and EB2. Duplicates were made for both samples.

Table 1: Concentration and amount of DNA samples

Sample	Concentration (ng/μl)	Amount (ng)
EB1	82.4	10
EB2	68.1	10

Table 2: Reaction set up for one PCR reaction with EB1 as DNA template

Component	Volume (μl)
Q5 High Fidelity 2X Master Mix	6.25
10 uM Forward Primer	0.63
10 uM EC_rev2	0.63
Template DNA	0.12
dH2O	4.9
Total Volume	12.5

Table 3: Reaction set up for one PCR reaction with EB2 as DNA template

Component	Volume (μl)
Q5 High Fidelity 2X Master Mix	6.25
10 uM Forward Primer	0.63
10 uM EC_rev2	0.63
Template DNA	0.15
dH2O	4.9
Total Volume	12.5

Results and Conclusion

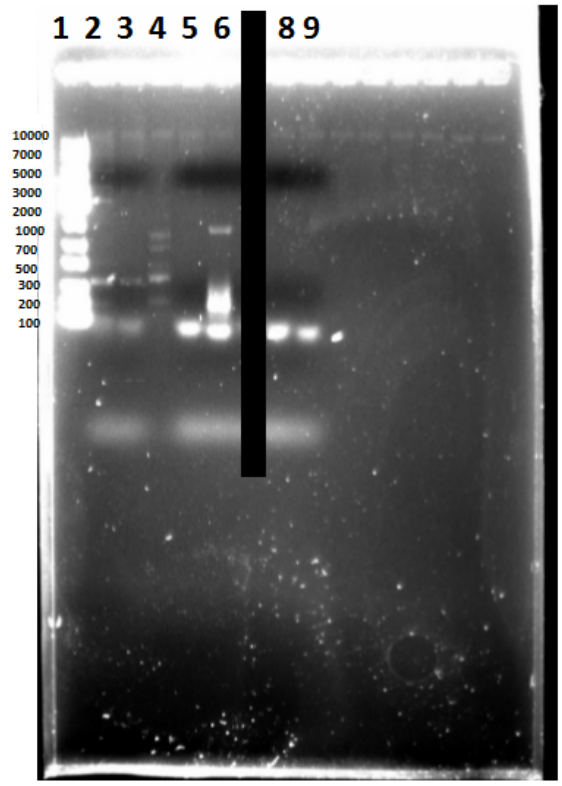

Figure 1: Results from gel electrophoresis for the overhang PCR.

Table 4: Order of the wells for the gel electrophoresis

Well	Sample
1	Ladder
2	EB1.1
3	EB1.2
4	Empty
5	EB2.1
6	EB2.2
8	Negative control
9	Negative control

As could be seen from the picture of gel in Figure 1, it's hard to distinquish the bands on the gel.

Discussion and Troubleshooting

The reason to the failure of the experiment was believed to be a mix up of the primers EC_rev2 and EB_rev2. EB_rev2 was supposed to be used for this experiment. A new trial will be made with EB_rev2 as primer and with increased amount of DNA. Furthermore, well 4 wasn't loaded but there is bands in that well, probably due to contamination. It was decided that no results could be trusted from this experiment.

2 Overhang PCR of T7-EB to add BamHI, second attempt

Responsible

Aman Mebrahtu, Oscar Frisell

Protocol Used

PCR protocol

Experimental Set Up

Description: Since last experiment failed because of the use of wrong primer, a new trial was made with the same conditions but with the right primer, EB_rev2. However, the amount of DNA used was increased from 10 ng to 20 ng. Gel electrophoresis was run at 120 V, 20 min. The concentration of the PCR products was measured with Nanodrop. The samples were diluted 1:10 after the Nanodrop measurement.

Table 5: Concentration and amount of the DNA samples

Sample	Concentration (ng/μl)	Amount (ng)
EB1	82.4	20
EB2	68.1	20

Table 6: Reaction set up for one PCR reaction with EB1 as DNA template

Component	Volume (μl)
Q5 High Fidelity 2X Master Mix	6.25
10 μM VF2	0.63
10 μM EB_rev2	0.63
Template DNA	0.24
dH2O	4.8
Total Volume	12.5

Table 7: Reaction set up for one PCR reaction with EB2 as DNA template

Component	Volume (μl)
Q5 High Fidelity 2X Master Mix	6.25
10 μM Forward Primer	0.63
10 μM EB_rev2	0.63
Template DNA	0.29
dH2O	4.7
Total Volume	12.5

Results and Conclusion

In figure 2 there are bands in well 2,3 and 4 slightly above 1000 bp which seems to correspond to the desired overhang PCR product.

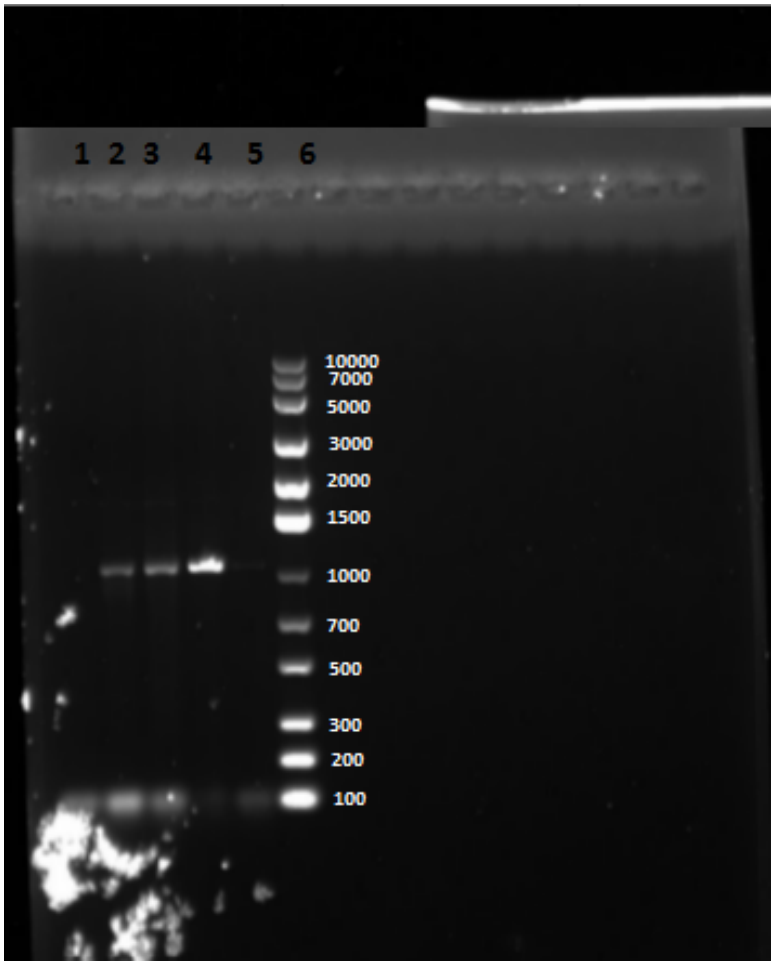

Figure 2: Results from gel electrophoresis for the overhang PCR.

Table 8: The order of the wells for the gel electrophoresis

Well	Sample
1	EB1.1
2	EB1.2
3	EB2.1
4	EB2.2
5	Negative control
6	Ladder

Table 9: Concentration of the PCR products

Sample	Concentration (ng/μl)	Concentration after dilution (ng/μl)
EB1.1	519.7	51.97
EB1.2	510.6	51.06
EB2.1	528.2	52.82
EB2.1	495.8	49.58

Discussion and Troubleshooting

This experiment was interpreted to be successful. It would be good to send the PCR products for sequencing but since that is time consuming we will move on and perform the digestion of the inserted BamHI-site.

3 Digestion of T7-EB-BamHI at the BamHI-site

Responsible

Aman Mebrathu

Protocol used

Digestion

Experimental Set up

Description: The aim of this experiment was to digest T7-EB-BamHI at the BamHI-site to get this BioBrick ready for ligation with Linker-tag. The digested samples were EB1.1 (52.82 ng/μl) and EB2.1 (51.97 ng/μl from last experiment. The samples were diluted 1:10 to get a final concentration of approximately 5 ng/ul.

Table 10: Reaction set up with EB1.1 or EB2.1 as DNA template

Component	Volume (μl)
10x Buffer B	1
BamHI	0.5
Sample DNA	0.96
dH2O	7.54
Total Volume	10

Results and Conclusion

No results found.

Discussion and Troubleshooting

Since no results have been documented it's assumed that this experiment failed.

4 Digestion of LT at BamHI-site to prepare it for ligation with T7-EB-BamHI

Responsible

Oskar Ohman

Protocol Used

Digestion
PCR Purification

Experimental Set Up

Description: First, the Linker (1613.4 ng/μl was digested at the BamHI-site. The reaction set up can be seen in Table 8. Three reactions were made. The digested 3 Linker-samples were pooled after PCR purification.

Table 11: Reaction set up for the digestion of Linker at BamHI-site

Component	Volume (μl)
DNA template	1
Buffer B	2
BamHI	1
dH2O	16
Total Volume	20

Results and conclusion

The concentration of the pooled LT-sample after PCR purification was 97.3 ng/μl.

Discussion and Troubleshooting

The Linker sample will be digested at the P-site and ligated with T7-EB-BamHI. Since it's not possible to see from a gel electrophoresis if this digestion works, because only a few nucleotides are cut, no analyse of the digestion result was made. It would have been preferable to send the sample for sequencing but since that is time consuming we choose to move on to next experiment.

5 Digestion of T7-EB-BamHI at BamHI and E-site and digestion of LT at P-site

Responsible

Oskar Ohman

Protocol Used

Digestion
Gel extraction
PCR Purification

Modifications and comments to the protocol

The idea was to gel purify the samples EB1.1 and EB2.1 and PCR purify the Linker after the digestion. By mistake EB1.1 was PCR purified instead.

Experimental Set Up

Description: Samples used for this experiment can be seen in Table 12.

Table 12: The concentration of the samples used

Sample	Concentration (ng/μl)
EB1.1	528.2
EB2.1	519.7
Linker	97.3

One digestion reaction was made for EB1.1 and EB2.1. Two digestion reactions for the Linker was made. The reaction set up can be seen in Table 13 and 14.

Table 13: Reaction set up using EB1.1 or EB2.1 as DNA template

Component	Volume (μl)
DNA template	3.82
EcoRI Mastermix	2
BamHI Mastermix	2
Buffer B	0.4
Total Volume	8.22

Table 14: Reaction set up with LT as DNA template

Component	Volume (μl)
DNA template	8.22
PstI Mastermix	2
Buffer H	0.6
Total Volume	10.82

The composition of the Enzyme mastermixes can be seen in Table 15.

Table 15: The composition of the enzyme mastermixes

EcoRI Mastermix		**BamHI Mastermix**		**PstI Mastermix**	
Component	Volume (μl)	Component (ul)	Volume (μl)	Component (μl)	Volume (μl)
EcoRI	4	BamHI	4	PstI	1.6
Buffer B	0.4	Buffer B	0.4	Buffer H	0.4
ddH2O	0	ddH2O	0	ddH2O	2
Total Volume	4.4	Total Volume	4.4	Total Volume	2.2

Results and Conclusion

Sample EB2.1 could not be detected on the gel. Therefore the gel extraction could not be done. In Table 16 the concentration of the PCR purified samples can be seen.

Table 16: Concentration of the digested samples after PCR purification.

Sample	Concentration (ng/μl)
EB1.1	4.6
Linker 1	16.2
Linker 2	18.1

Discussion and Troubleshooting

The vision is to create the construct pSB1C3-T7-EB-Linker with the PCR-purified samples in next experiment.

6 Ligation of T7-EB and LT into pSB1C3 and transformation into DH1-α cells

Responsible

Oskar Ohman, Aman Mebrahtu, Sigrun Stulz

Protocols Used

Ligation
Transformation

Experimental Set Up

Table 17: Samples used for the ligation

Sample	Concentration (μl)
EB1.1	4.6
Linker 2	18.1
pSB1C3	12.7

Table 18: Reaction set up for one reaction. One reaction was made

Component	Volume (μl)
EB1.1	15
Linker 2	0.33
pSB1C3	3.94
T4 Ligase	0.5
10x T4 DNA Ligase Buffer	3
ddH2O	7.2
Total Volume	30

Results and Conclusions

No cells were found on the Agar-plate.

Discussion and Troubleshooting

The experiment failed. Another attempt will be made with no heatkill and longer incubation time. (To see details about how this experiment was performed see the ligation protocol)

7 PCR amplification of pSB1C3-T7-EB

Responsible

Aman Mebrathu, Reskandi Rudjito

Protocol Used

PCR with Q5

Experimental Set Up

Description: The aim of this experiment was to amplify sample EB1.1 to get enough DNA to be able to do a second attempt creating the pSB1C3-T7-EB-Linker construct.

Table 19: Reaction set up for one PCR reaction with EB1.1 as DNA template.

Component	Volume (μl)	Final concentration
Q5 High-Fidelity 2X Master Mix	6.25	1X
10 μM Forward primer	0.63	0.5 μM
10 μM Reversed primer	0.63	0.5 μM
Template DNA	0.5	1000 ng
Nuclease-Free water	4.5	
Total volume	12.5	

Results and conclusions

No results found.

Discussion and Troubleshooting

Since no results have been documented the experiment is assumed to have been unsuccessful.